REMARKS

The amendments to claims 1 and 8 are simply for clarity as discussed at the interview. Support for the inclusion of 1,500 bases as an upper limit defining the target region is found in paragraph 32 on page 10. The insertion of "identification" before probes provides antecedent basis for this term in claims 7 and 14 and is supported by paragraph 31. The term "oligomer" has been changed to "oligonucleotide" for clarity. The limitation that the identification be observed on a single copy of a target nucleic acid is supported in paragraph 11. The remainder of the claim has been amended to emphasize that the particulate labels are, as was previously required, visualized as "separate points in space." As kindly suggested by Examiner Forman at the interview, the concluding phrase "interrogating said region to genotype said target nucleic acid" has been added consistent with the title of the case and with the descriptions, for example, in paragraph 11, and as exemplified in the following paragraphs – *e.g.*, measuring the length of repeats or SNP's as set forth in paragraphs 14 and 15. Claim 8 is simply for identification of a target nucleic acid and is supported in paragraphs 33, 34 and 35.

As the amendments are for clarification only, no new matter has been added and entry of the amendment is respectfully requested.

The Invention

The invention offers a simple, convenient and sensitive alternative to conventional methods of genotyping. It can be performed on a mixture of nucleic acids (see paragraph 44) and detects a single copy of nucleic acid without the need for amplification (see paragraph 11). In one aspect, this is enabled by using particulate labels to identify and bracket a region of a single copy of nucleic acid molecule to be interrogated (see paragraph 12) and directly observing and interrogating the

region using a microscope. Other nucleic acids may be present on the microscopically observed surface, but only a single copy of the nucleic acid to be interrogated need be observed.

The interrogation can take a variety of forms, but in all cases the identifying bracketing labels focus the attention of the observer on the appropriate location for observation. Without this focus, the interrogation must be made more specific than that permitted by the identification probes of the invention.

For example, as noted in paragraph 18, an SNP can be directly detected by using a labeled probe that is so small (*e.g.*, a 5-mer) that it will fail to bind unless there is a perfect match – so the binding or non-binding of the probe in the identified region gives an immediate answer as to whether the SNP is present. Of course, many regions in a genome would bind such a small probe, so that the results of supplying the probe alone to a nucleic acid sample would show a multiplicity of locations where the probe is bound. But by bracketing the region of interest by identification probes, the observer knows to look at binding only in the relevant region.

Other ways to interrogate the identified region include testing for the presence or absence of a restriction site – in its presence, treatment of the sample with the restriction enzyme results in separation of the previously paired labels. In this case, one of the labels can be supplied attached to a probe that further includes the complementary strand to the restriction site (see paragraph 17).

Because of the various procedures by which an identified region can be interrogated, and because the step of identifying the region to be interrogated in the manner required by the invention is unique, claim 1 and its dependent claims, as presented, focus on the identification (bracketing) step, *per se*.

In another aspect, represented by claim 8 and its dependent claims, the use of two labels of different hues, each coupled to a probe associated with adjacent regions of a target nucleic acid, can serve simply to determine whether a particular nucleic acid is present in a sample (see paragraph 34). Again only a single copy need be observed. Because two beads are used, specificity is greatly increased and the assay is easily multiplexed.

The Rejections

Applicants appreciate the withdrawal of some rejections previously made. The outstanding rejections are addressed as follows:

The Rejection of Claims 1-5 and 7-17 as Anticipated by Bawendi, *et al.* (US 6,306,610)

The cited portions of Bawendi themselves clarify that there is no anticipation. The claims have been amended to emphasize that the particulate labels must be individually identified as separate points in space. This is clear from the specification as well, as noted on page 10 – in lines 2, *et seq*. This portion of the specification states that the spacing must be sufficient to accommodate the apparent swelling of the beads, so that they are spaced sufficiently that they do not blend.

This is in contrast to the description in Bawendi - figure 3 and column 5, lines 40-45, state that the probes are hybridized to DNA sequences in close proximity and detected by fluorescence resonance energy transfer (FRET). The other section referred to by the Office in column 24 is under the heading of "FRET" in line 21. In FRET, the particulate labels are not observed individually as separate points in space. Rather, presence or absence of quenching is observed. That is, the only thing that can be observed is whether there is a brighter or dimmer emission of

fluorescence. In view of the failure of Bawendi to disclose the limitation of the claim that the particulate labels be observed separately, it cannot anticipate.

In the case of claim 1, Bawendi further does not anticipate because no step of interrogating a bracketed region is described.

Indeed, the focus of Bawendi is apparently elsewhere in designing suitable forms of quantum dots that might be useful in biological assays in general.

Because Bawendi does not anticipate the independent claims, the dependent claims are free of this rejection as well and the rejection may be withdrawn.

The Rejection of Claim 6 as Assertedly Obvious Over Bawendi in View of Nie, *et al.* (US 6,060,242)

This basis for rejection is moot in view of the patentability of the independent claim from which claim 6 depends.

The Rejection of Claims 1-3, 5, 7-10, 12, 14-15 and 17 as Obvious Over Gray, *et al.* (US 6,475,720) in View of Barbera-Guillem, *et al.* (US 6,307,701)

The Office asserts that it would be obvious to the skilled artisan to modify the method of Gray by using a nucleic acid probe coupled to the particulate labels described by Barbera-Guillem. The basis for this rejection is that the properties of these labels would be known to be superior to the fluorophores employed by Gray, and applicants appreciate the detailed response to their previous argument. In reconsidering this combination, applicants realize that it is not necessary to revert to an assertion of hindsight to argue this rejection, as it is apparent to them that the combination of Gray with Barbera-Guillem, even if made, does not result in the invention as now claimed.

The procedure described by Gray, even if modified to substitute the particulate labels which are the subject of Barbera-Guillem for the fluorophores employed by Gray, would not result in the invention. In the method of the invention, the nucleic acids exist as independent single copy molecules in the samples, *i.e.*, they are present in isolated form in the sample. The procedure described by Gray is an *in situ* hybridization (FISH) which requires the DNA to be present in its context in the chromosome. In column 4, this is made clear at line 47,

> When the target nucleic acid remains in its natural biological setting, *e.g.*, DNA in chromosomes, mRNA in cytoplasm, portions of chromosomes or cell nuclei (albeit fixed or altered by preparative techniques) the hybridization process is referred to as *in situ* hybridization.

The claims do not relate to *in situ* hybridization, but rather hybridization conducted where the nucleic acids are isolated from their native environment and observable as single copies.

Second, and perhaps more important, the methods of claims 1 and 8 require "oligonucleotide" probes. The precise length of oligonucleotides is not defined in the art, but it is clearly a relatively short stretch of bases; some definitions note as few as 20. As noted by Gray *et al.*, their method requires probes "in an approximate complexity range of from about 50,000 bases (50 kb) to hundreds of millions of bases" (column 6, line 56). In the particular experiment cited by the Office, the combined length of the probes is indeed about 50 kb. As set forth beginning at the bottom of column 58, the ABL probe is a 35 kb cosmid and the BCR probe is an 18 kb phage clone. Neither of these would be considered "oligonucleotides."

Further, the proximity envisioned even in CML subjects when the probes are to be observed "together" is at a minimum of 25 kb (column 61, line 8), whereas, as noted in the specification, the

regions of the nucleic acid being identified are separated, in general, by distances of less than/about 1.5 kb.

In short, the procedure set forth in Gray does not really resemble the method set forth in the invention and which is the subject of the claims. The method of Gray is conducted *in situ* in the chromosome simply to discern whether portions of the chromosomes are spliced together. Single copies of nucleic acid molecules are not observed. The invention method is performed on isolated DNA – *i.e.*, outside of the context of its usual environment in the chromosomes which would include protein as well as nucleic acids. The method of Gray requires very large oligonucleotide probes of the order of 50 kb, whereas the method of the invention, because it uses isolated single copies of nucleic acids, requires only oligonucleotide probes. Further, in claim 1, the step of interrogating the bracketed region is required and no such interrogation is suggested by Gray. In view of these differences, substituting the label of Barbera-Guillem falls short of suggesting the presently claimed method. For this reason, this basis for rejection may properly be withdrawn.

The Remaining Rejections

Claims 4, 6, 11 and 13 were rejected on the same basis but with the addition of Nie, and claim 16 with the addition of Ward. These rejections are now moot in view of the foregoing distinctions between the combination of Gray and Barbera-Guillem in the independent claims from which these claims depend.

Conclusion

Applicants wish, once again, to express their appreciation to Examiners Shaw and Forman for their helpful discussion at the interview.

The outstanding rejection for anticipation over Bawendi, *et al.,* may be withdrawn since Bawendi does not disclose separate identification and observation of the particulate labels employed. Bawendi is limited to fluorescence quenching, which does not permit separate observation of the two probes. The rejection over Gray in combination with Barbera-Guillem may be withdrawn because the method of Gray, even if modified by substituting the labels described by Barbera-Guillem, does not result in or suggest the method as claimed. Gray's method is conducted *in situ* and employs probes with nucleotide sequences at least two orders of magnitude greater than those employed in the present invention. Single copies of nucleic acid molecules are never observed. The observation of the association of the labels in Gray is over a range of at least 25 kb, a range which is more than an order of magnitude greater than the distance of bracketing associated with the present invention and specified now in the claims. Therefore, applicants believe claims 1-17 are in a position for allowance and passage of these claims to issue is respectfully requested.

Should there be remaining issues that might be resolved by phone, a telephone call to the undersigned is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 388512011000.

Respectfully submitted,

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